New Zealand Journal of Zoology, 2006, Vol. 33: 189–196
0301–4223/06/3303–189

A new species *Macrobiotus magdalenae* (Tardigrada: Eutardigrada: Macrobiotidae, *richtersi* group) from Costa Rican rain forest (Central America)

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Abstract A new species *Macrobiotus magdalenae* sp. nov. is described from a liverwort sample collected in Costa Rica (Central America). The new species is most similar to *Macrobiotus corgatensis* Pilato et al., 2002 but differs from it mainly by the lack of fine granulation on the cuticle.

Keywords Tardigrada; Costa Rica; new species; taxonomy; *Macrobiotus magdalenae* sp. nov.; *M. corgatensis*; *M. richtersi* group

INTRODUCTION

Our knowledge of the tardigrades of Costa Rica is extremely poor. Only 17 species have been reported from this country so far (Kaczmarek 2003; Kaczmarek & Michalczyk 2004; Michalczyk & Kaczmarek 2006). This paper describes and figures a new species of the genus *Macrobiotus* Schulze, 1834 collected in Costa Rica.

The new species belongs to the *richtersi* group, which until now has consisted of 12 species (in alphabetical order): *Macrobiotus alekseevi* Tumanov, 2005, *Macrobiotus chieregoi* Maucci & Durante Pasa, 1980, *Macrobiotus corgatensis* Pilato et al., 2002, *Macrobiotus danielae* Pilato et al., 2001, *Macrobiotus garynahi* Kaczmarek et al., 2005, *Macrobiotus gerlachae* Pilato et al., 2004, *Macrobiotus lorenae* Biserov, 1996, *Macrobiotus peteri* Pilato et al., 1989, *Macrobiotus priviterae* Binda et al., 2001, Macrobiotus *richtersi* Murray, 1911, *Macrobiotus savai* Binda & Pilato, 2001 and *Macrobiotus vanescens* Pilato et al., 1991. All known species of this group lay areolated eggs with truncoconical/cone-shaped processes, and have three elongated macroplacoids and a microplacoid placed far from the third macroplacoid. However, even though it is not certain whether the eggs of *M. chieregoi* have areolation, Pilato (2001) included this species into the *richtersi* group due to the buccal apparatus morphology (Pilato pers. comm.). If further research proves that eggs of *M. chieregoi* are not areolated it should probably be excluded from the *richtersi* group.

MATERIAL AND METHODS

Seventeen specimens of the new species and seven of its eggs were found in a liverwort sample collected in Costa Rica in December 2002. Fifteen specimens and four eggs were mounted on microscopic slides in Hoyer's medium; two adults and three eggs were prepared for Scanning Electron Microscopy (SEM).

All measurements are given in micrometers (μm). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). Buccal tube width was measured as the external diameter at the level of the stylet support insertion point. External and internal claws were measured. Lengths of the claw branches were measured from the base of the claw to the top of the branch, including accessory points. The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981). In the description of the new species,

Z06003; Online publication date 5 July 2006
Received 24 January 2006; accepted 14 April 2006

Fig. 1 *Macrobiotus magdalenae* sp. nov. 1, Habitus (adult). (DIC)

the *pt* is given after the µm value, in square brackets and italics. Terminology describing the oral cavity armature is given according to Michalczyk & Kaczmarek (2003).

Photomicrographs and drawings were made using Nomarski Differential Interference Contrast Microscope (DIC) and SEM.

TAXONOMIC ACCOUNT OF THE NEW SPECIES

***Macrobiotus magdalenae* sp. nov.** (Fig. 1–29)

MATERIAL EXAMINED: 17 adults, and 7 eggs (prepared for both DIC and SEM) from the type locality.

DESCRIPTION: Adult (measurements of the holotype): Body length 515.0 (Fig. 1). Body transparent/white. Eyes present, elongated (photosensory cells arranged longitudinally, Fig. 29). Cuticle smooth, without pores. Granulation present only on legs IV.

Bucco-pharyngeal apparatus of *Macrobiotus* type (Fig. 2–12). Mouth antero-ventral, surrounded by ring of 10 peribuccal lamellae. Oral cavity armature with three bands of teeth (Fig. 5–12).

Teeth of first band (Fig. 5, 7, 11–12): these are smaller than those of the other two bands and are in the shape of small granules. They are present in anterior portion of oral cavity just behind peribuccal lamellae and also on them. This first band of teeth is continuous and looks the same on all oral cavity walls.

The second band of teeth (Fig. 5–12): these are intermediate in size between those of the first band and those of the third band of teeth. They are in the shape of small ridges parallel to the main axis of the buccal tube (ventral teeth are little larger then dorsal). They are positioned in the posterior portion of the oral cavity just behind the ring fold and just before the third band of teeth. The second band of teeth is continuous and arranged in one row. In smaller animals most teeth are uniform and regular, in the shape of ridges, but in larger specimens teeth are very often joined one-by-one. Joined teeth are H-, V-, and W-shaped.

The third band of teeth (Fig. 5–12): these are larger than those in the other two rows and usually number six to nine. They are mostly in the shape of transverse ridges/baffles. Teeth in this band are positioned in the rear of the oral cavity just behind the second band of teeth and just before the buccal tube opening. Usually this band is not continuous and is divided into two series, ventral and dorsal. Dorsal series consists of three bar-shaped teeth (one median and two lateral, Fig. 9–12). Ventral series consists of 1–4 median and two lateral, rectangular or rounded teeth (Fig. 5–8). Medio-ventral teeth may be arranged in two rows (anterior and posterior). The anterior row contains a higher number of teeth than the posterior one (Fig. 6–7).

Buccal tube 57.0 long and 11.5 [*20.2*] wide with one bend in anterior part of tube (visible in lateral view). Stylet supports inserted on buccal tube at

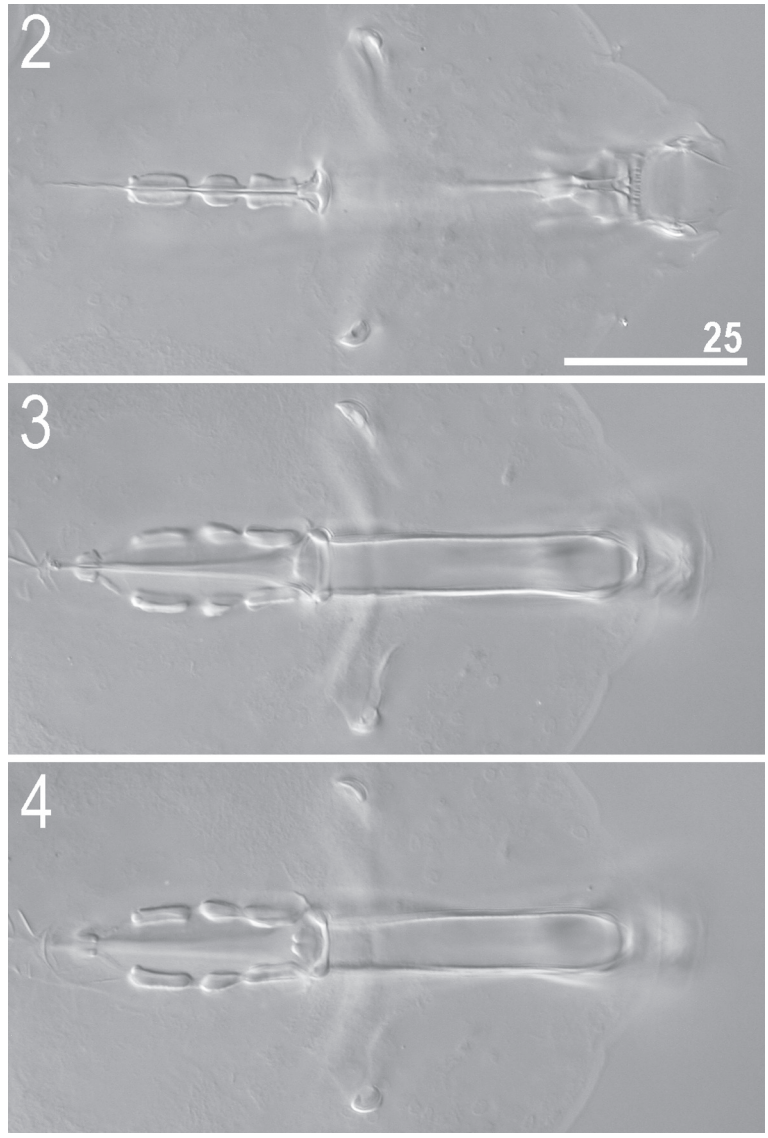

Fig. 2–4 *Macrobiotus magdalenae* sp. nov. Buccal apparatus: 2, ventral view (note ventral teeth of the second and third band); 3, mid section (note the stick-like structure between the third macroplacoids and the microplacoids); 4, dorsal view. (DIC)

47.0 [*82.5*]. Pharyngeal bulb slightly oval with apophyses, three macroplacoids and microplacoid (Fig. 2–4). Pharyngeal apophyses distinct, triangular, narrower and indented posteriorly. First macroplacoid thinner anteriorly, 10.0 [*17.5*] long, second oval, 8.0 [*14.0*] long, both without constriction. Third macroplacoid 12.0 [*21.1*] long, with constriction in subterminal part. Microplacoid 4.0 [*7.0*] long (Fig. 3). Microplacoid connected with third macroplacoid by thin cuticular stick-like structure (Fig. 3–4). Macroplacoid row 34.0 [*59.6*] long. Placoid row 42.5 [*74.6*] long.

Claws of *hufelandi* type, slender with narrow bases (Fig. 13–14). Primary branches with distinct accessory points. Lunules on all legs smooth, better developed on IV pair of legs. Primary branch of external claw (pb) of legs I 12.0 [*21.1*] long, secondary branch (sb) 9.0 [*15.8*] long; II pb 13.0 [*22.8*], II sb 10.0 [*17.5*] long; III pb 13.0 [*22.8*], sb 10.0 [*17.5*]; IV pb 14.5 [*25.4*], sb 11.0 [*19.3*]. Bars on legs absent.

Eggs: White, laid freely (Fig. 15–28). Spherical, areolated, with 10–12 processes on circumference. Processes in the shape of cones, elongated in

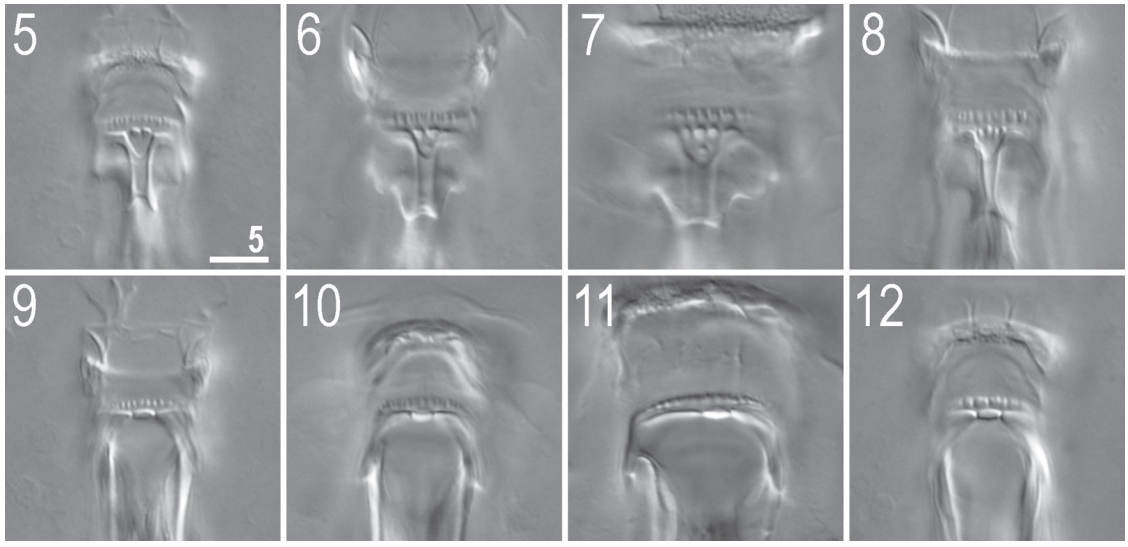

Fig. 5–12 *Macrobiotus magdalenae* sp. nov. Oral cavity armature: 5–8, ventral side (5, third band of teeth with two medio-ventral teeth; 6, with three teeth arranged in two rows; 7, with four teeth arranged in two rows; 8, with four teeth arranged in one row); 7–12, dorsal side (5 and 9, 6 and 10, 7 and 11, 8 and 12 are the same buccal apparatuses). (DIC)

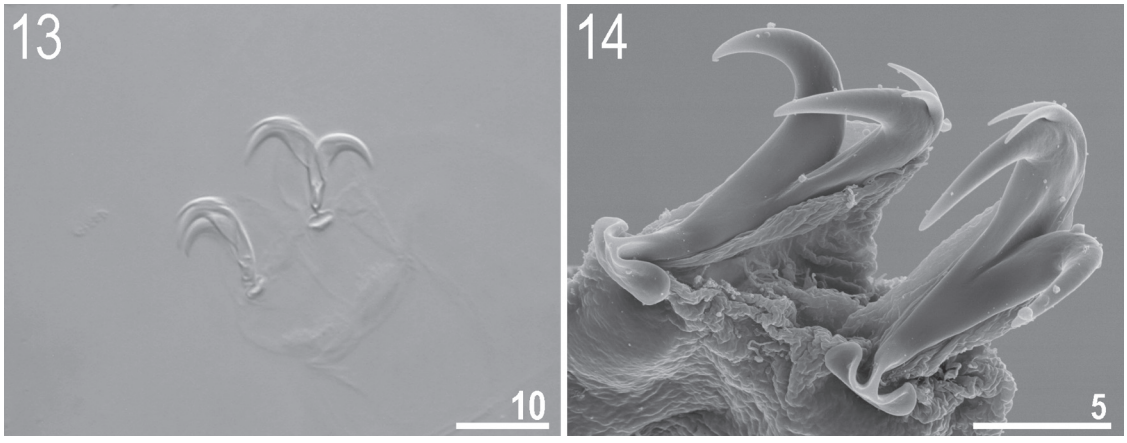

Fig. 13–14 *Macrobiotus magdalenae* sp. nov. Claws of the III pairs of legs. (13, DIC; 14, SEM)

terminal part, however the shape is variable (Fig. 19–26). Elongated terminal part covered with irregular granules, and may be very short (Fig. 24), or thin and long (Fig. 22, 26), or split in the end (Fig. 23). Processes consist of double wall with transverse supporting walls forming "cells" visible in DIC as a dense reticular design ("reticulation" slightly elongated vertically) (Fig. 19). External walls of processes smooth or slightly wrinkled (wrinkles in form of rings around a process), without pores. Internal walls strongly porous (visible in SEM only). Surface between processes areolated (Fig. 15, 17–18, 25–28). Areolae oval in DIC (Fig. 15) and polygonal in SEM (Fig. 27–28). Each areola with an irregular thickened, porous central portion (pores are not always visible in DIC) (Fig. 27–28). Number of areolae around a process depends on number of processes surrounding particular process. Usually, there are two areolae per each neighbour process, but sometimes only one develops. Therefore, if five

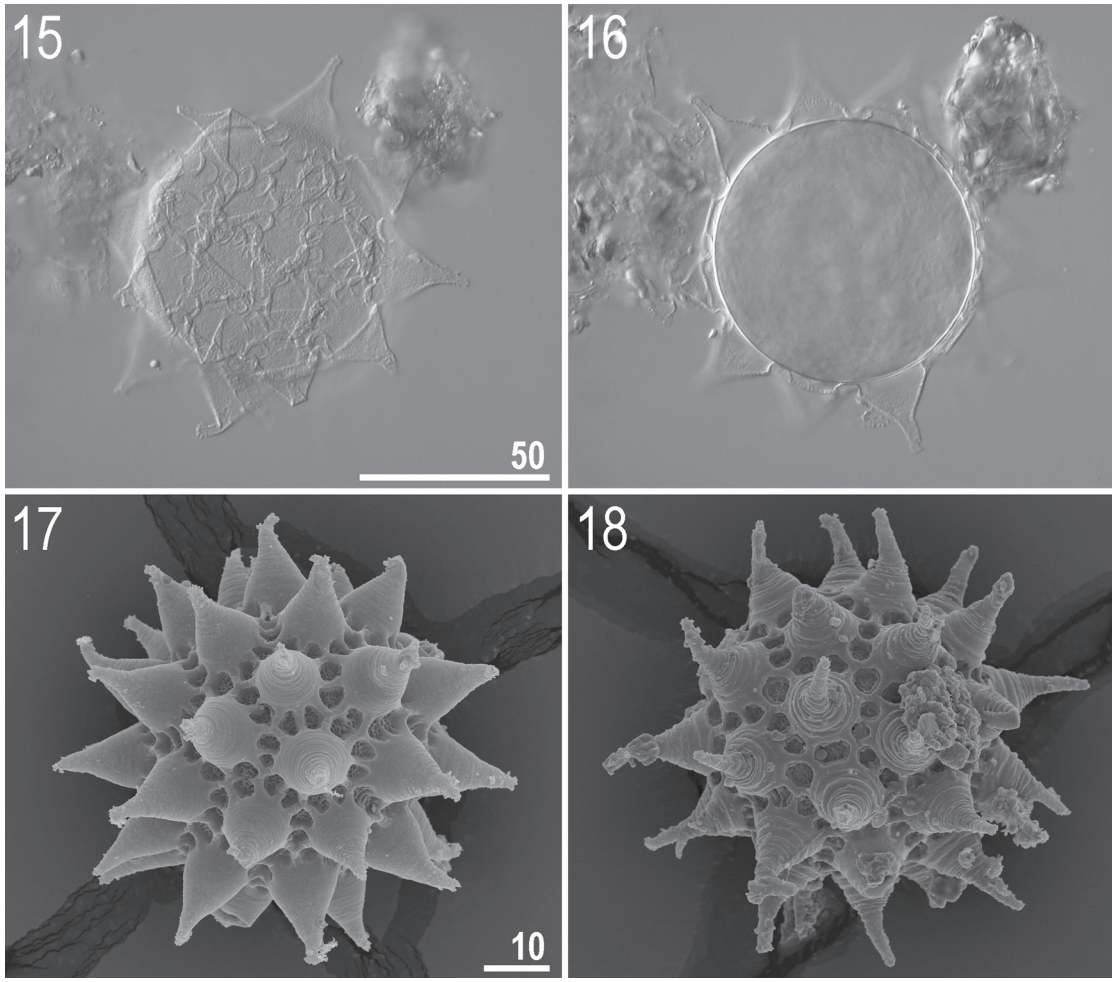

Fig. 15–18 *Macrobiotus magdalenae* sp. nov. Eggs. (15–16, DIC; 17–18, SEM)

processes surround particular process, usually 8–10 areolae are present (maximum 10). If 6 processes surround particular process, usually 9–12 areolae are present (maximum 12) (Fig. 17–18).

REMARKS: Adults: Results of simple statistical analysis of measurements and *pt* values of selected morphological structures for six specimens are given in Table 1. Four of 14 (29%) specimens (mounted in Hoyer's medium) do not have eyes. Eggs: Statistics for all measurable eggs are provided in Table 2.

TYPE LOCALITY: Costa Rica, Heredia Province, Tropical wet forest, liverwort from tree, "La Selva" Biological Station (the trail "SAT-500"); 30 Dec 2002; leg. Ł. Kaczmarek.

TYPE DEPOSITORIES: Holotype and 18 paratypes (14 adults and 4 eggs) are deposited in the Natural History Collections, Faculty of Biology, A. Mickiewicz University, Umultowska 89, 61–614 Poznań, Poland.

ETYMOLOGY: The new species is dedicated to the first author's mother, Magdalena Piąsta.

DIFFERENTIAL DIAGNOSIS: *Macrobiotus magdalenae* sp. nov. is the thirteenth described species in the *richtersi* group (the twelfth if *M. chieregoi* does not belong to the group). The species within the group are usually most easily distinguished using characters of the egg shell surface. Eggs can be divided into two groups in terms of the areolation

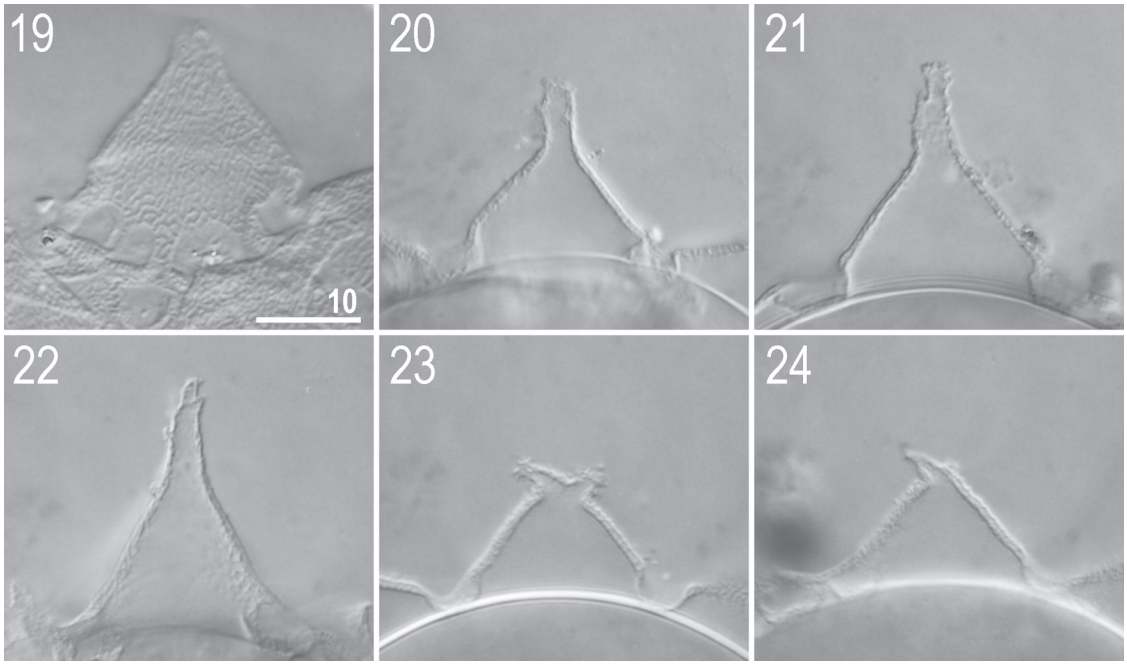

Fig. 19–24 *Macrobiotus magdalenae* sp. nov. Variability of egg processes' shapes and sizes: (19, surface of the process with "reticulation"; 20–24, mid sections). (DIC)

Table 1 Measurements (in µm) and *pt* values of selected morphological structures of specimens of *Macrobiotus magdalenae* sp. nov. mounted in Hoyer's medium. Range refers to the smallest and the largest structure found among all measured specimens; *N*, number of specimens/structures measured. SD, Standard deviation.

		Range		Mean		SD	
Character	*N*	(µm)	(*pt*)	(µm)	(*pt*)	(µm)	(*pt*)
Body	14	205.0–545.0	*766.7–999.0*	427.9	*892.1*	93.4	*64.7*
Buccal tube	14	24.0–57.0	–	47.7	–	8.4	–
Stylet support insertion point	14	18.5–47.0	*77.1–82.5*	38.4	*80.4*	7.1	*1.3*
Buccal tube external width	14	4.0–12.0	*16.1–22.2*	9.0	*18.8*	2.2	*1.7*
Macroplacoid 1	13	3.0–10.0	*12.5–17.5*	7.3	*15.3*	1.8	*1.5*
Macroplacoid 2	13	2.0–8.0	*8.3–14.3*	5.4	*11.1*	1.7	*1.9*
Macroplacoid 3	13	3.0–12.0	*12.5–21.1*	8.7	*18.0*	2.3	*2.3*
Microplacoid	13	2.0–5.0	*5.7–8.9*	3.3	*6.9*	0.8	*0.9*
Macroplacoid row	13	10.0–34.0	*41.7–60.0*	24.1	*50.3*	6.5	*5.7*
Placoid row	13	13.0–42.5	*54.2–76.4*	31.1	*65.0*	8.1	*6.7*
Claw 1—primary branch	12	8.0–12.5	*20.0–33.3*	10.8	*23.2*	1.4	*3.5*
Claw 1—secondary branch	12	5.0–9.0	*14.9–20.8*	8.0	*17.1*	1.3	*1.7*
Claw 2—primary branch	14	8.0–14.0	*21.8–33.3*	11.2	*23.9*	1.7	*3.1*
Claw 2—secondary branch	14	5.0–9.0	*15.6–20.8*	8.4	*17.7*	1.4	*1.4*
Claw 3—primary branch	10	9.0–13.0	*21.8–26.7*	11.9	*23.6*	1.4	*1.6*
Claw 3—secondary branch	10	7.0–10.0	*16.4–20.0*	9.0	*17.8*	1.0	*1.1*
Claw 4—primary branch	5	11.5–14.5	*25.0–27.7*	13.5	*26.1*	1.3	*1.1*
Claw 4—secondary branch	5	8.5–11.0	*18.9–19.6*	10.0	*19.3*	1.2	*0.3*

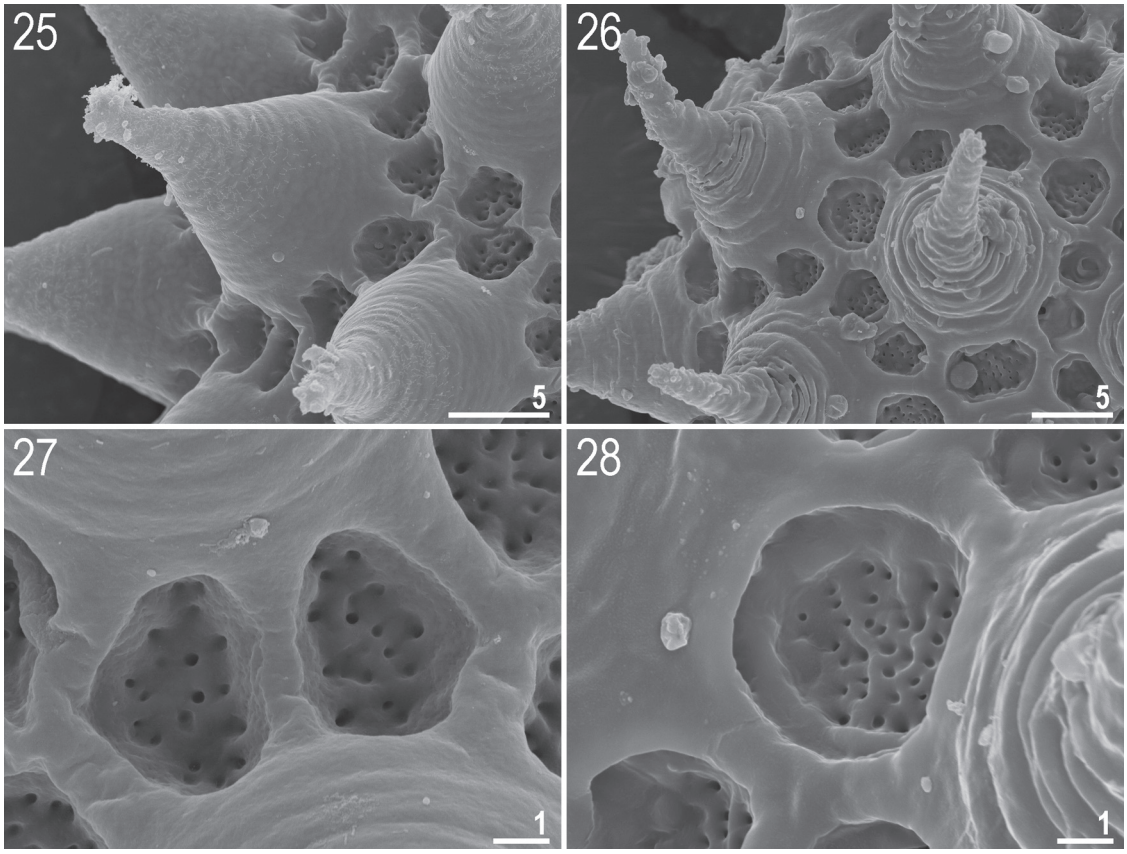

Fig. 25–28 *Macrobiotus magdalenae* sp. nov. Egg processes and areolation. (SEM)

Table 2 Measurements (in µm) of selected morphological structures of eggs of *Macrobiotus magdalenae* sp. nov. mounted in Hoyer's medium. Minimum and maximum refer to the smallest and the largest structure found among all measured eggs; *N*, number of eggs/structures measured.

Character	*N*	Minimum	Maximum	Mean	SD
Diameter of egg without processes	4	70.0	74.0	71.5	1.9
Diameter of egg with processes	4	102.0	115.0	108.0	5.7
Processes height	15	13.0	25.0	18.0	3.3
Processes base width	15	2.0	21.0	17.1	4.6
Number of processes on the circumference of egg	4	10	12	11.0	0.8

type: the central portion of areolae may be either smooth, or thickened and sculptured/porous. The first described species with areolae having a smooth central portion was *M. richtersi*, and the first species with areolae having a sculptured central areolae was *M. vanescens* (Pilato et al. 1991). Therefore, we use the following terms to describe the areolation type: *richtersi*-type and *vanescens*-type. Among the species of the *richtersi* group described so far there are five species with the *vanescens*-type areolation: *M. alekseevi*, *M. corgatensis*, *M. gerlachae*, *M. priviterae*, and *M. vanescens*.

In the appearance of the egg shell, the new species is most similar to *M. corgatensis* Pilato et al., 2002

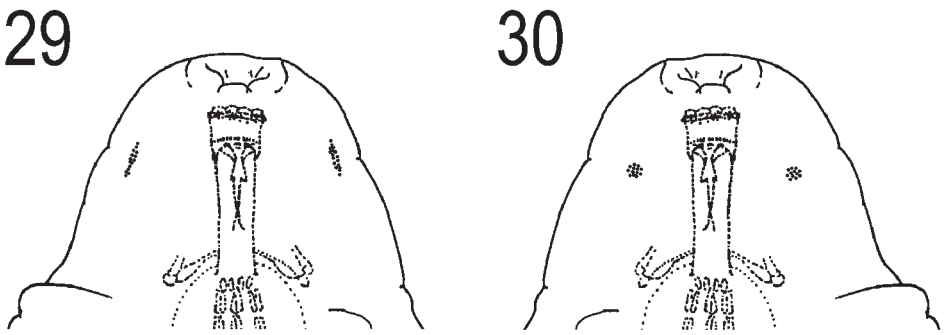

Fig. 29–30 29, *Macrobiotus magdalenae* sp. nov.—elongated eyes; 30, *Macrobiotus corgatensis* Pilato et al., 2002—round eyes (Fig. 30 from Pilato et al. 2002, modified; the body's contour in Fig. 29 also taken from Pilato et al. 2002).

from the Seychelles, but the eggs of *M. corgatensis* are slightly larger in diameter (70.0–74.0 in the new species and 75.0–90.0 in *M. corgatensis*). Moreover, adults of *M. magdalenae* sp. nov. differ from adults of *M. corgatensis* by: having smooth cuticle (fine granulation present on the whole cuticle in *M. corgatensis*) and smooth lunulae on all claws (small teeth present on lunulae of the IV pair of claws in *M. corgatensis*). Eyes in *M. magdalenae* sp. nov. are longitudinally elongated, while they are rounded in *M. corgatensis* (Fig. 29–30) (Pilato et al. 2002).

ACKNOWLEDGMENTS

The authors are grateful to the reviewers, Clark W. Beasley (USA) and Peter Degma (Slovakia) for the valuable comments to the manuscript. This research was partially supported by the Instituto Nacional de Biodiversidad (INBio) and the Ministry of Environment and Energy (MINAE) with funds from the Global Environment Facility (GEF) through the World Bank and project ALAS with funds from National Science Foundation (Grant no. DEB-0072702). The work was also partially supported by a grant to the first author from the European Commission's programme Transnational Access to Major Research Infrastructures to COBICE (Copenhagen Biosystematics Centre) and by a grant to both authors from the European Commission's programme Transnational Access to Major Research Infrastructures to SYNTHESYS (Grant no. DK-TAF-1163).

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